Arch. Pharm. Res. Vol.19, No.5, pp. 429-431, 1996

# A Novel Cycloartane Glycoside from *Thalictrum uchiyamai*

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*(Received August 3, 1996)*

A new cycloartane glycoside (**1**) was isolated from the aerial part of *Thalictrum uchiyamai* Nakai (Ranunculaceae). On the basis of chemical and physicochemical evidence, the aglycone structure of this compound was characterized as 16,25-dihydroxy-3,24-diacetoxy-9, 19-cycloartane-29-oic acid, a new derivative of cycloartane triterpene. Also, the oligosaccharide moiety of this glycoside were determined as 29-*O*-α-L-rhanmnopyranosyl-(1→2)-[β-D-xylofuranosyl-(1→6)]-β-D-glucopyranose by application of HMBC technique. Consequently, the structure of compound 1 was elucidated as 29-*O*-α-L-rhanmnopyranosyl-(1→2)-[β-D-xylofuranosyl-(1→6)]-β-D-glucopyranosyl-16,25-dihydroxy-3,24-diacetoxy-9,19-cycloartane-29-oic acid ester.

**Key words:** Cycloartane triterpene, 16,25-Dihydroxy-3,24-diacetoxy-9,19-cycloartane-29-oic acid, Glycoside, *Thalictrum uchiyamai*

## INTRODUCTION

*Thalictrum* species are used as drugs for the treatment of diarrhea, cuts and bruise in Japan (Hasegawa, 1969), and anti-inflammatory agent in China (Chung and Shin, 1990). *Thalictrum uchiyamai*, an indigenous plant in Korea, are seldom found around in Seoul area. In continuing study on the active constituent of *T. Uchiyamai* (Lee and Lee, 1977; Lee, 1984), we wish to report the isolation of a new cycloartane triterpene glycoside from the *n*-BuOH fraction.

## RESULTS AND DISCUSSION

The *n*-butanol extract of the aerial parts of *Thalictrum uchiyamai* was subjected to column chromatography on Sephadex LH-20 and silica gel to afford compound 1.

The negative FAB mass showed quasimolecular ion peak at *m/z* 1029 $[M-H]^+$ which, together with $^{13}$C-NMR data, suggested the molecular formula as $C_{51}H_{82}O_{21}$. The signal appeared at *m/z* 589 was assigned to aglycone peak. Compound **1** was suspected to contain a cyclopropyl ring system, as a result of the observation of two doublets appearing at δ 0.27 ppm (1H, *J* =4.8 Hz), 0.71 (1H, *J*=4.8 Hz) in its $^1$H-NMR spectrum and the characteristic resonances seen in its $^{13}$C-NMR spectrum at 30.20 ppm ($CH_2$, C-19) (Choi *et al.*, 1989).

Spectral evidence was obtained for the presence of acetate group in the molecule of compound **1**, including the ir absorption band at 1730 $cm^{-1}$, and methyl singlets at δ 2.05 and 2.02 ppm in the $^1$H-NMR spectrum. Of the downfield signals in the $^1$H-NMR spectrum of **1**, resonances at 4.76, 4.59 ppm were assigned to the methine protons adjacent to acetoxyl groups, and these were shifted upfield by about 1.4 ppm (to δ 3.29, 3.19 ppm, respectively) in the compound **2**, the aglycone which was produced from **1** by treatment with KOH. The proton appeared at δ 4.30 ppm in compound **1** were assigned to methine proton geminal to hydroxyl group which did not shifted (δ 4.34) in the $^1$H-NMR spectrum of compound **2**. All of these peaks were shifted downfield to δ 4.57 and 5.11 ppm in the compound **2a** which was produced from compound **2** by treatment with acetic anhydride in pyridine.

The position of two acetoxyl and a hydroxyl group was proposed as C-3, C-24 and C-16, respectively, by comparison of $^{13}$C-NMR spectra of compound **1** and **2**. The $^{13}$C-NMR data of compound **2** indicated that signals of C-2 (+4.22 ppm) and C-25 (+0.95 ppm) was deshielded due to the deacetylation effect. Spectral evidence obtained by careful examination of $^1$H-$^1$H COSY, $^1$H-$^{13}$C HMQC, and $^1$H-$^{13}$C HMBC spectra of compound **1** further confirmed these substitution pattern. The appearance of two methyl group at δ 1.13 (6H, s) suggested that a hydroxyl group was present at the C-25 position.

A carbon peak appearing at δ 172.90 in the $^{13}$C-NMR spectra of compound **1** was assigned to C-29 by comparison of $^{13}$C-NMR data with those of abrusoside A (Choi *et al.*, 1989), and confirmed by observing a

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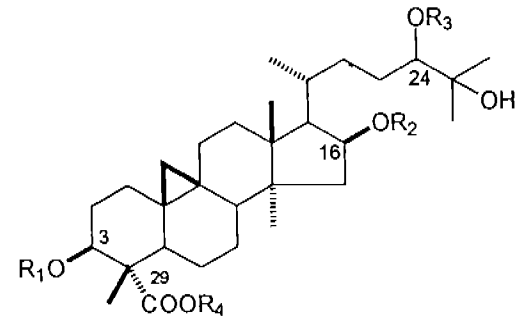
contour spot between C-29 and H-3 in the $^{1}H$-$^{13}C$ HMBC spectrum. Thus, the aglycone structure of compound **1** was characterized as 16,25-dihydroxy-3,24-diacetoxy-9,19-cycloartane-29-oic acid, a new derivative of cycloartane triterpene.

The proton signals appearing at δ 4.19 (1H, $J$=7.3 Hz), 5.29 (1H, $J$=1.6 Hz) and 5.56 (1H, $J$=7.6 Hz) ppm were assigned to the anomeric protons, and corresponding carbon signals were found at δ 105.59, 101.52 and 94.57 ppm, respectively, by performing $^{1}H$-$^{13}C$ HMQC experiment. The sugars units and the configurations at the anomeric centers of compound **1** were established as β-D-xylopyranose, α-L-rhamnopyranose, and β-D-glucopyranose by acid hydrolysis and the $J_{1,2}$ couplings of anomeric protons (7.3, 1.6, and 7.5 Hz, respectively), and their $^{13}C$-NMR data.

Comparison of the $^{13}C$-NMR data due to aglycone moiety of compound **1** with those compound **2** indicated that C-29 signal in **1** appeared at higher field (-5.97 ppm) indicating that glycosylation took place at the C-29 position. Examination of $^{13}C$-NMR data in the sugar carbon region of compound **1** showed the downfield shifts of glucose C-2', and C-6', suggesting the presence of sugar units at these position. This result was further verified by observing cross peaks between the aglycone C-29 and glucose H-1', C-2' and rhamnose H-1", and C-6' and xylose H-1"' signals in the $^{1}H$-$^{13}C$ HMBC spectrum. Thus, the saccharide linkage in the compound **1** was determined as α-L-rhanmnopyranosyl-(1→2)-[β-D-xylofuranosyl-(1→6)]-β-D-glucopyranose.




**1** $R_1$, $R_3$ = Ac; $R_2$ = H; $R_4$ = glc(2→1)rha, glc(6→1)xyl

**2** $R_1$, $R_2$, $R_3$, $R_4$ = H

**2a** $R_1$, $R_2$, $R_3$ = Ac; $R_4$ = H

## General experimental procedures

Melting points were determined using a Mettler FP 62 instrument and are uncorrected. The uv spectra were obtained on a Shimadzu UV-240 spectrophotometer and ir spectra were measured on a Perkin-Elmer 1420 spectrophotometer. $^{1}H$- and $^{13}C$-NMR spectra were recorded on a Bruker AMX-500 spectrometer. FAB mass spectra were obtained with a VG 70V SE spectrometer. HPLC was conducted on a JASCO Familic-300 system with a Waters RI detector, and a Waters carbohydrate column (3.9 id × 30 cm).

## Plant material

The aerial parts of *Thalictrum uchiyamai* Nakai (Ranunculaceae) were collected in Dobong mountain, Seoul in June 1991.

## Extraction and fractionation

The air-dried plant material (1.8 kg) were extracted with hot MeOH three times, and gave 230 g of a dried MeOH extract on removal of solvent *in vacuo*. The water soluble portion of this residue was extracted successively with *n*-hexane, ethyl ether, and *n*-BuOH, and the BuOH-soluble portion was concentrated. The residue (40 g) was dissolved in MeOH, and was purified by gel filtration with Sephadex LH-20 using MeOH, and chromatography with silica gel using $CHCl_3$-$CH_3OH$-$H_2O$ (45 : 15 : 1) to afford compound **1**.

Compound **1**-colorless powders; Rf 0.47, $CHCl_3$-$CH_3OH$-$H_2O$ (15:7:1); Rf 0.58, EtOAc-MeOH-$H_2O$ (10:3:2); Rf 0.16, benzene:ethyl formate:formic acid (1:5:2); mp 220-222°C; uv (EtOH) end absorption; ir $\nu_{max}$ (KBr) 3410, 2920, 1730, 1450, 1250, 1070, 1020 $cm^{-1}$; $^{1}H$-NMR (500 MHz, $CD_3OD$) δ 0.27, 0.71 (2H, d, $J$=4.8 Hz, 19-H2), 0.87 (3H, s, 28-$CH_3$), 0.88 (3H, d, $J$=6.5 Hz, 21-$CH_3$), 1.12 (3H, s, 18-$CH_3$), 1.13 (6H, s, 26,27-$CH_3$), 1.19 (3H, d, $J$=6.2 Hz, 6"-$CH_3$), 1.26 (3H, s, 30-$CH_3$), 2.02 (3H, s, $OCOCH_3$), 2.05 (3H, s, $OCOCH_3$), 4.19 (1H, d, $J$=7.3 Hz, 1"'-H), 4.30 (1H, m, 16-H), 4.59 (1H, dd, $J$=5.0, 11.8 Hz, 3-H), 4.76 (1H, br. d, 24-H), 5.29 (1H, d, $J$=1.6 Hz, 1"-H), 5.56 (1H, d, $J$=7.6 Hz, 1'-H) ppm; $^{13}C$-NMR (125 MHz, $CD_3OD$) δ 173.36 (24-Ac), 172.90 (C-29), 172.66 (3-Ac), 105.59 (C-1'"). 101.52 (C-1"), 94.57 (C-1'), 81.37 (C-24), 81.33 (C-3), 79.16 (C-3'), 77.56 (C-2'), 77.53 (C-3"') 77.02 (C-5'), 74.88 (C-2"'), 73.79 (C-4"), 72.89 (C-16), 72.86 (C-25), 72.11 (C-3"), 70.99 (C-4"'), 70.80 (C-4'), 70.04 (C-5"), 69.68 (C-6'), 66.81 (C-5"') 57.81 (C-17), 51.47 (C-4), 49.98 (C-8), 49.56 (C-15), 49.34 (C-5), 47.73 (C-14), 46.48 (C-13), 34.11 (C-22), 33.80 (C-12), 32.34 (C-1), 31.06 (C-20), 30.20 (C-19), 28.00 (C-2), 27.61 (C-7), 27.49 (C-11), 27.30 (C-23), 26.80 (C-10), 25.89 (C-26), 25.84 (C-27), 23.24 (C-6), 22.08 (C-9), 21.87 (C-30), 21.38 (24-Ac), 21.18 (3-Ac), 20.62 (C-18), 20.01 (C-28), 18.42 (C-6"), 18.21 (C-21) ppm; FAB-MS *m/z* $[M-H]^+$ 1029, $[aglycone-H]^+$ 589, $[aglycone-H-42]^+$ 547, $[aglycone-H-60]^+$ 529.

## Acid hydrolysis of compound 1

Compound **1** (20 mg) was hydrolyzed by treatment

with 5% $H_2SO_4$ under reflux on a water bath for 3 hrs. The precipitates were filtered off, and remaining aqueous solution was concentrated and analyzed by HPLC, which revealed the presence of glucose (Rt=7.2), rhamnose (Rt=4.0), and xylose (Rt=4.8).

### Alkaline hydrolysis of compound 1

Compound **1** (150 mg) was hydrolyzed with 1 M KOH under reflux on a water for 4 hrs. The reaction mixture was then acidified to pH 3 with 1 N HCl, and extracted with *n*-BuOH. The BuOH extract was washed with water and concentrated to give residue, which was chromatographed on silica gel with $CHCl_3$-$CH_3OH$ (10:1) to give compound **2** (50 mg).

Compound **2**-colorless powder, Rf 0.44, $CHCl_3$-$CH_3OH$-$H_2O$ (9:2:0.2) uv (EtOH) end absorption; ir $\nu_{max}$ (KBr) 3410, 2960, 1470, 1450, 1390, $cm^{-1}$; $^1$H-NMR (500 MHz, $CD_3OD$) δ 0.24, 0.55 (2H, d, *J*=4.4 Hz, 19-$H_2$), 0.84 (3H, s, 28-$CH_3$), 0.86 (3H, d, *J*=6.5 Hz, 21-$CH_3$), 1:06 (3H, s, 27-$CH_3$), 1.08 (H, s, 26-$CH_3$), 1.09 (H, s, 18-$CH_3$), 1.26 (3H, s, 30-$CH_3$), 3.19 (1H, dd, *J*=4.7, 11.8 Hz, 3-H), 3.29 (1H, dd, *J*=2.2, 10.9 Hz, 24-H), 4.34 (1H, dt, *J*=5.0, 10.3 Hz, 16-H) ppm; $^{13}$C-NMR (125 MHz, $CD_3OD$) δ 179.33 (C-29), 79.21 (C-24), 78.45 (C-3), 73.81 (C-25), 73.36 (C-16), 58.13 (C-17), 52.53 (C-4), 50.08 (C-8), 49.46 (C-5), 49.28 (C-15), 47.73 (C-14), 46.44 (C-13), 34.06 (C-22), 33.71 (C-12), 33.04 (C-1), 32.22 (C-2), 30.36 (C-19), 29.84 (C-20), 28.46 (C-23), 27.62 (C-10), 27.52 (C-7), 27.37 (C-11), 25.42 (C-26), 25.37 (C-27), 23.86 (C-6), 22.78 (C-30), 22.60 (C-9), 20.57 (C-18), 19.67 (C-28), 18.43 (C-21) ppm.

### Acetylation of compound 2

Compound **2** (30 mg) was acetylated overnight at room temperature in pyridine-$Ac_2O$ (1:1). The reaction mixture was partitioned between $Et_2O$ and water, and $Et_2O$ layer was washed with water and concentrated to give residue, which was chromatographed on silica gel with $CHCl_3$-$CH_3OH$ (15:1) to give compound **2a** (10 mg).

Compound **2a**-colorless powder, Rf 0.43, $CHCl_3$-$CH_3OH$ (15:1); uv (EtOH) end absorption; $^1$H-NMR (500 MHz, $CD_3OD$) δ 0.30, 0.48 (2H, d, *J*=4.7 Hz, 19-$H_2$), 0.85 (3H, d, *J*=5.9 Hz, 21-$CH_3$), 0.87 (3H, s, 28-$CH_3$), 1.05 (3H, s, 27-$CH_3$), 1.05 (3H, s, 26-$CH_3$), 1.07 (3H, s, 18-$CH_3$), 1.11 (3H, s, 30-$CH_3$), 1.89 (3H, s, $OCOCH_3$), 1.93 (3H, s, $OCOCH_3$), 1.96 (3H, s, $OCOCH_3$), 4.57 (2H, m, 3,24-H), 5.11 (1H, m, 24-H) ppm.

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